GENETIC EVIDENCE OF ABERRANT CHROMOSOME BEHAVIOR IN MAIZE ENDOSPERM[1]

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The occasional appearance of a maize seed, the endosperm of which is in part colored and in part colorless or in part starchy and in part sugary, has long been known, and much speculation has been indulged in by geneticists in attempts to account for the phenomenon. Some years ago the writer (Emerson, 1915) reviewed the hypotheses that had been previously offered as possible explanations of such seeds and suggested the further hypothesis of somatic mutation, a suggestion that has been repeated, apparently independently, by J. L. Collins (1919). It was noted also that irregular chromosome behavior might possibly be concerned. In a later paper (Emerson, 1918) numerous cases of anomalous endosperm development were reported and discussed in relation to the hypotheses of somatic mutation and of aberrant chromosome behavior. It was pointed out that the facts then at hand could be accounted for equally well by either of the two hypotheses, and the kind of evidence necessary for a crucial comparison of the two was noted.

In the latter paper evidence was presented that tended to prove that aberrant seeds are not produced (1) when the dominant endosperm factor concerned, for example the aleurone-color factor *C*, is homozygous and therefore triplex, *C C C*, or (2) when the dominant factor is brought into the cross by the female parent and its recessive allelomorph by the male parent, *C C c*, but only (3) when the dominant factor is contributed by the male alone, *c c C*. In the case of either *C C C* or *C C c*, a single mutation from the dominant to its recessive allelomorph could result only in *C C c* or *C c c*, respectively, and the aleurone would still be colored and no apparent anomaly would result. To produce colorless aleurone, *c c c*, two mutations in case of *C C c* and three in case of *C C C* must occur simultaneously or successively in the endosperm of the same seed—a chance so small that it might well be disregarded. It was noted that a single dominant mutation from *c* to *C* should change colorless, *c c c*, to colored, *C c c*, aleurone, but the relative infrequency of dominant mutations was thought to account for the lack of observed aberrant seeds in homozygous colorless types.

Similarly, it was noted that if a single non-disjunction of the chromosome carrying *C* or *c* occurred, it could not result in a visible change in aleurone color in case of such genotypes as *C C C*, *C C c*, or *c c c*, but only

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with *c c C*. In the latter case, if the chromosome carrying *C* failed to divide or if the two halves failed to separate after division, one of the resulting daughter nuclei would be *c c C* or *c c C C* (colored) and the other *c c* (colorless). Thus both somatic mutation and chromosome non-disjunction might readily account for the observed cases of aberrant endosperm, and neither mutation nor non-disjunction could reasonably be expected to cause such an anomaly in genotypes where it has never been observed.

It was pointed out in the writer's 1918 paper that crucial evidence in support of one or other of these hypotheses might be obtained only from crosses in which linked aleurone and endosperm factors are simultaneously involved. It was known that the aleurone factor pair *C c* is thus linked with waxy endosperm, *Wx wx* (Bregger, 1918; Kempton, 1919), but no aberrant seeds positively known to involve both these factor pairs were available. The writer was not unaware of G. N. Collins's case (1913) involving the aleurone factor pair *I i* with *Wx wx*, but the linkage relations of these factors were not known. It has since been shown by Hutchison (1921) that the factor pair *I i* is closely linked with a factor pair for shrunken endosperm, *Sh sh*, which in turn is linked with *C c* and *Wx wx*. The linkage group as at present known, therefore, is made up of the pairs *C c*, *I i*, *Sh sh*, and *Wx wx*. Consequently, in accordance with the chromosome hypothesis, all these factor pairs are assumed to lie in one pair of homologous chromosomes.

Assuming, then, that *C* and *Wx* lie in the same chromosome, it can readily be seen how a crucial test of the somatic-mutation and the chromosome-non-disjunction hypotheses is afforded by appropriate crosses. If the female parent of a cross be colorless and waxy, *c wx*, and the male parent be colored and corneous, *C Wx*, the resulting endosperm will be *c c C wx wx Wx*, and the three homologous chromosomes carrying these genes in the "fecundated" endosperm nucleus will be as follows:

1	*c*	*wx*
2	*c*	*wx*
3	*C*	*Wx*

DIAGRAM 1

Now if, at any division of an endosperm nucleus, the two halves (*a* and *b*) resulting from a longitudinal split of the chromosome carrying *C* and *Wx* (chromosome 3 of diagram 1) should fail to separate (non-disjunction) and should go together to one pole (*A*), the resulting daughter nuclei (*A* and *B*) would be as shown in diagram 2.

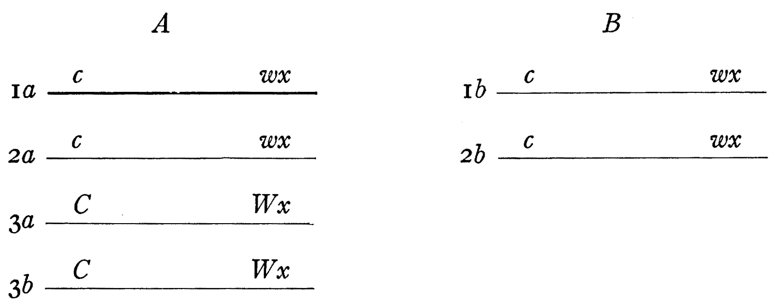

DIAGRAM 2

Obviously nucleus "*B*" and all its descendants would lack both *C* and *Wx* so that the resulting aleurone would be colorless and its underlying endosperm waxy, while the aleurone and endosperm cells resulting from the further division of nucleus "*A*" would be colored and corneous. The same results would follow if chromosome 3 failed to divide, going entire to one pole, or if after equational division one of the halves were left behind, failing to reach either pole.

If, on the other hand, the colorless part of an aberrant seed be due to a somatic mutation of *C* to *c*, there is no reason to suppose that the same mutation would change *Wx* to *wx*. From what is known of the origin of factor ("point") mutations, there is little if any more warrant for the assumption that a single mutation will ordinarily involve simultaneously two loci of one chromosome than that it will affect loci of non-homologous chromosomes. If, therefore, in the case under consideration, the colorless part of an aberrant seed be due to a somatic mutation, the endosperm underlying it should be corneous, *c c c wx wx Wx*, like that underlying the colored part of the aleurone, *c c C wx wx Wx*.

It now remains to examine the evidence derived from crosses of colorless waxy individuals, *c Wx*, with pollen of colored corneous ones, *C Wx*, and to determine whether the colorless parts of aberrant seeds resulting from such crosses are underlaid with waxy or with corneous endosperm. The data available from the writer's cultures are presented in table 1. Of the 65 aberrant seeds there recorded, the part with colorless aleurone was underlaid by waxy endosperm in 55 cases, by corneous endosperm in 3 cases, and in the remaining 7 cases the endosperm texture could not be determined either because of the extremely small size of the colorless spots or because of the immaturity of the seeds.

The aberrant parts of these seeds varied in area from not much more than a square millimeter to about two thirds of the entire surface of the seed, 32 of the 65 seeds having one sixth or more and only 4 having more than one half of the surface colorless. Eight of the 65 seeds had numerous colorless spots of varied sizes but mostly small, and all the others had only a single spot each. The line of demarcation between the colored and color-

less parts was invariably sharp but usually somewhat irregular. The correspondence in outline between the colorless aleurone and the underlying waxy endosperm was strikingly exact irrespective of the number of spots per seed or of their irregularity (fig. 1; *I*, *J*, *K*, *L*). The waxy endosperm was found to extend to varying depths, the smaller spots often being more shallow than the larger ones (fig. 1; *K*, *L*). Moreover, the larger waxy parts often exhibited a somewhat irregular outline in cross-section (fig. 1, *K*). It is perhaps possible that the three seeds noted as having corneous endosperm under the colorless aleurone had in reality a very shallow layer of waxy endosperm, but this is not likely since in neither case did the colorless spot include less than about one fourth of the entire area of the seed.

The writer has examined three aberrant seeds involving *C c* and *Wx wx* from cultures other than his own and in each case the colorless aleurone was directly over waxy endosperm. The seed described by G. N. Collins (1913), from F_2 of a cross of white waxy with pollen of colored non-waxy types, in which the colorless aleurone was underlaid by waxy and the colored part by corneous endosperm, involved, it is now almost certain, *C c* with *Wx wx*. Collins's published F_2 records leave no doubt that he was dealing with a case of linkage between waxy endosperm and some aleurone factor. The cross certainly did not involve the aleurone factor pair *I i*, for the colorless condition was recessive. Aleurone-color factors *A a* (Bregger, 1918) and *R r* (Kempton, 1919) are now known to be inherited independently of *Wx wx*, so that *C c* is the only known factor pair that could have been involved. Since, however, Collins's case appeared in F_2 and since there is about 25 percent of crossing-over between *C c* and *Wx wx*, there is no certainty that both *C* and *Wx* were in one chromosome and *c* and *wx* in another.

The evidence derived from crosses involving the linked genes *C-Wx* and *c-wx* points conclusively—in so far as genetic evidence can be regarded as at all conclusive with respect to cytological behavior—to some aberrant chromosome distribution, perhaps non-disjunction, as the cause of most cases of aberrant endosperm development; but the three instances noted above of corneous endosperm underlying colorless spots of aleurone suggest, though they do not prove, that very rarely somatic mutation may be responsible.

Evidence from other linked factors in addition to *C c* and *Wx wx* would be of great value as tending to confirm or contradict the conclusion here drawn. A number of such linkages are now known. In addition to the linkage of *I i* with *Wx wx*, inferred, as noted earlier in this paper, from Hutchison's data, Hutchison has found both *I i* and *C c* to be closely linked with shrunken endosperm, *Sh sh*, and Dr. E. G. Anderson (unpublished data) has noted linkage between a factor pair for blotched aleurone, *Bh bh*, and the pair *Y y* for yellow endosperm.

The writer has not been able as yet to obtain from his own cultures aberrant seeds involving any of these linkages, but in Professor Hutchison's material a single aberrant seed involving *C c* and *Sh sh* has been observed. A colorless- and shrunken-seeded plant, *c sh*, pollinated by a plant heterozygous for these factors, *C c Sh sh*, produced the aberrant seed. It was

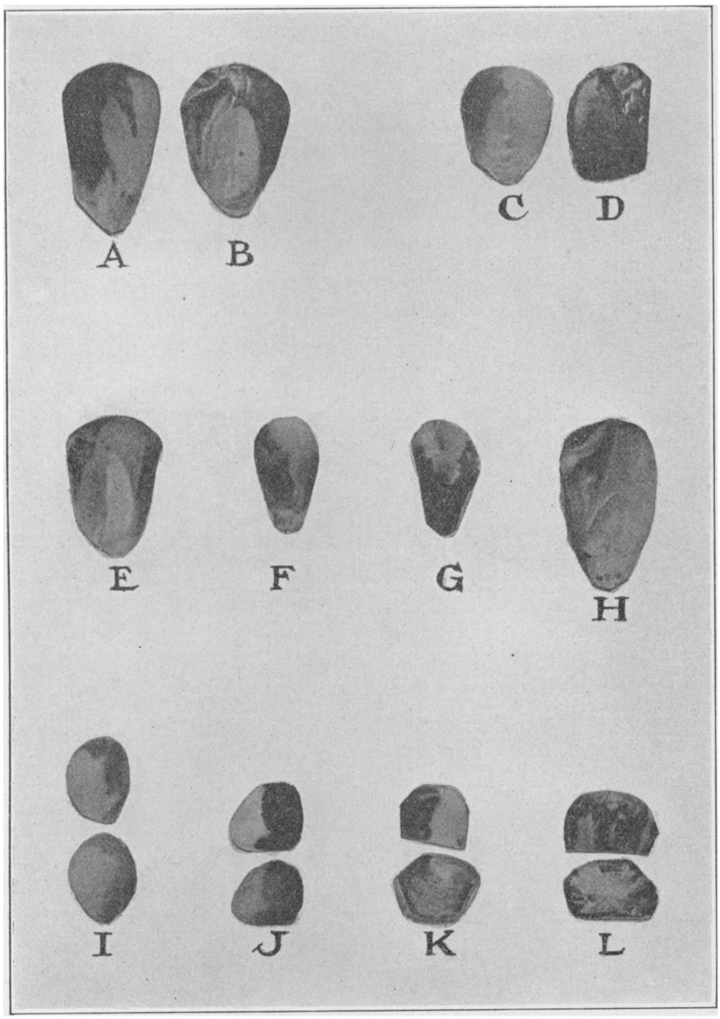

FIG. 1. Aberrant endosperm in maize seeds. Factors involved: A and B, *R r Su su;* C and D, *C c Su su;* E, *R r Wx wx;* F, *A a Wx wx;* G, *C c Sh sh;* H, *Su su;* I to L, *C c Wx wx.* In I and J the upper figures show untreated seeds with purple-colorless aleurone, and the lower figures show the same seeds after the pericarp and aleurone layer have been removed, the corneous endosperm appearing dark and the waxy endosperm light. In K and L, the upper figures represent parts of untreated seeds and the lower figures corresponding cross sections of the same seeds.

colored and non-shrunken, *C Sh*, except for a single large spot that was both colorless and shrunken (fig. 1, G). The sperm in this case must have carried both *C* and *Sh*, for otherwise the seed would have been colorless and shrunken throughout. The evidence, therefore, so far as this one seed is concerned, is definitely in favor of the hypothesis of aberrant chromosome behavior and opposed to that of somatic mutation.

Both *I i* and *Wx wx* are without doubt concerned in the case of a single aberrant seed reported by G. N. Collins (1913). A colored waxy type pollinated by a colorless corneous one resulted in colorless corneous seeds. The dominance of the colorless condition establishes, so far as is now known, the presence of *I* in the colorless pollen parent. A single seed of this cross, though colorless and corneous in the main, had a small spot of colored aleurone which overlaid exactly a spot of waxy endosperm. It seems evident that in this one instance in which an aberrant seed involved the linked factors *I* and *Wx*, just as in the single case in which *C* and *Sh* were involved and in the great majority of the cases—55 out of 58—in which the linked factors *C* and *Wx* were concerned, aberrant endosperm development is ordinarily due to some unusual chromosome behavior possibly of the nature of non-disjunction.

One unfamiliar with some of the results previously published will not have failed to observe by this time that either one of Webber's (1900) well-known hypotheses might account for the results presented above quite as well as the hypothesis of non-disjunction. Webber, it will be recalled, suggested as possible explanations of aberrant endosperm development (1) that the second sperm nucleus on the one hand and the fused polar nuclei on the other may occasionally develop independently, each giving rise to a part of the endosperm, or (2) that the second sperm nucleus may sometimes unite with one polar nucleus, leaving the other polar nucleus to develop independently. If either of these things should happen, it is obvious that, in cases of aberrant endosperm where *C* and *Wx* come from the male and *c* and *wx* from the female parent of a cross, the colored parts must be corneous and the colorless ones waxy. The second sperm nucleus, carrying *C* and *Wx*, whether it divide independently or unite with one polar nucleus, would give rise to colored corneous endosperm, and the endosperm developed either from one polar nucleus alone or from a fusion of the two, both carrying *c* and *wx*, would produce colorless waxy endosperm.

But it was shown by East (1913) that Webber's first hypothesis, independent development of the sperm nucleus and of the fused polar nuclei, was untenable. Crosses were made between two types of maize, both with colorless aleurone but one having factor *C* and the other having factor *R*, both of which are essential to aleurone-color development. Among the numerous colored seeds resulting from these crosses, six were colored on one side and colorless on the other. It is obvious that these aberrant seeds could not have arisen in accordance with Webber's first hypothesis, for,

since the second sperm nucleus carried *C* and not *R* and the polar nuclei *R* and not *C*, if the two elements divided without undergoing fusion, *C* and *R* could never have been brought together and no aleurone color could have developed in any part of the seeds.

TABLE I. *Aberrant seeds of maize from crosses of colorless waxy, c wx, by colored corneous, C Wx*

Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds. Endosperm under Colorless Parts		
		Waxy	Corneous	Undetermined
9062– 2	460	2		
3	600	3		
3	500	1		
6	280	1	1	
9064– 2	10	1		
9491– 1	560	1		1
2	250	1		
10168– 5	480	1		
10169– 3	430	1		
10170– 1	400	2		
2	300	2		
2	460	1		
4	390	4		1
5	310			1
7	70	1		
10171– 1	460	2		1
4	450	1		
6	650	1		
7	620	2	1	
8	340	2		
10	430	2		
11	290	1		
11	540	1		
13	160	1		
14	560	3		
16	530	1		
17	270	1		
18	630	3		
18	470	2		
19	190	2		
23	240	1		
24	350	2		
25	140	1		
27	470	2		
29	430			1
10182– 1	150			2
5	240	1	1	
11	120	1		
29 ears	9,580	0	0	0
Total 67 ears	23,810	55	3	7

In a similar way the writer (Emerson, 1915) was able to show that Webber's second hypothesis is incorrect. Two colorless types, each having only one of the two complementary aleurone-color factors *C* and *R*, were crossed as in the experiments of East. In addition, the type used as the female parent was sugary and the one used as the male parent was starchy.

Among the resulting seeds, which were colored and starchy as expected, occurred two with aberrant endosperm, one of which was part colored and part colorless but starchy throughout and the other one part starchy and part sugary but colored throughout. As in the case reported by East, no color could have developed if the second sperm nucleus had not fused with a polar nucleus. Furthermore, if the second sperm nucleus had united with one polar nucleus (Webber's second hypothesis), the part of the endosperm so formed must have been both colored and starchy while the remaining part of the endosperm formed from the other polar nucleus alone must have been both colorless and sugary. The observed facts, namely, that the starchy-sugary seed was colored throughout and that the colored-colorless one was starchy throughout, indicated clearly that normal fusion of the second sperm nucleus with the polar nuclei had taken place.

It remains to forestall the justifiable criticism that one or other of Webber's hypotheses might still account for most examples of anomalous endosperm, the two seeds noted above being minor exceptions, just as either of these hypotheses might well be used to explain the 55 aberrant seeds with colorless spots waxy recorded in table 1, the 3 seeds with colorless spots corneous likewise being exceptions. In the writer's 1918 paper (tables 8 and 10) were recorded 33 examples of anomalous seeds with part colored and part colorless aleurone from crosses between types with wholly colorless aleurone but carrying complementary aleurone-color factors. These, added to the six cases reported by East (1913), a total of 39, are believed to suffice as a demonstration that division of the second sperm nucleus independently of the fused polar nuclei is quite untenable. Moreover, two of the 33 anomalous seeds afforded definite evidence against the hypothesis that one polar nucleus might fuse with the second sperm nucleus and the other polar nucleus divide independently, making a total of four such instances. These two seeds resulted from a cross of a type with colorless sugary endosperm and colorless aleurone carrying *R* with pollen of a type with yellow starchy endosperm and colorless aleurone carrying *C*. The aberrant seeds were starchy and yellow throughout but their aleurone was about half colorless and half purple.

Since the publication of the writer's 1918 paper, a sufficient mass of evidence has been obtained to remove, it is thought, any possibility of explaining anomalous endosperm development on the basis of a failure of normal fusion of the second sperm nucleus and the polar nuclei. This additional evidence is presented in tables 2 and 3.

In table 2 is recorded all the available material in which corneous and waxy endosperm, *Wx wx*, are involved together with the aleurone-factor pairs *A a*, *R r*, and *Pr pr*. The male parents of all the crosses here recorded had homozygous corneous endosperm and homozygous purple aleurone, *A C R Pr Wx*, while the female parents of all had waxy endosperm, *wx*. In addition, the female parents of all crosses recorded in groups 1 and 2 of

the table had colorless aleurone, *a C R* for group 1 and *A C r* for group 2, and those of the crosses shown in group 3 had red aleurone, *A C R pr*. In all, 38 aberrant seeds are reported, 12 of which involved *A a Wx wx* (fig. 1, F), 6 *R r Wx wx* (fig. 1, E), and 20 *Pr pr Wx wx*. In all these cases,

TABLE 2. *Aberrant seeds of maize from crosses of colorless waxy, a wx and r wx, by colored corneous, A R Wx, and of red waxy, pr wx, by purple corneous, Pr Wx*

Group	Genes Concerned	Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds, all Corneous
				Colored and colorless
1..........	*a wx* × *A Wx*....	9061– 3	300	2
		5	500	1
		10183– 2	500	2
		8	350	3
		11	520	1
		14	280	2
		15	240	1
		12 ears	4,610	0
Total.......		19 ears	7,100	12
2..........	*r wx* × *R Wx*....	10187– 5	290	1
		6	440	1
		8	240	2
		9	190	2
		2 ears	560	0
Total.......		6 ears	1,720	6
				Purple and red
3..........	*pr wx* × *Pr Wx* ..	9062– 2	460	1
		5	470	1
		10171– 4	450	1
		6	650	1
		9	590	1
		14	560	1
		15	540	2
		18	630	2
		18	470	1
		23	280	1
		27	470	1
		10182– 1	390	1
		2	300	1
		5	480	2
		9	390	1
		10183– 7	280	1
		10187– 6	440	1
		8	240	1
		6 ears	2,220	0
Total.......		24 ears	10,310	20

the recessive aleurone color of the female parent, namely, colorless in groups 1 and 2 and red in group 3, occurred in the aberrant part of the seed, and the dominant color, purple, of the male parent occurred in the normal part. But in every instance the endosperm was corneous throughout like that of

the male parent. No case of aberrant corneous-waxy endosperm was observed, but there is no satisfactory evidence that none occurred among the wholly colored seeds, where waxy spots would be easily overlooked.

Similarly, in table 3 are recorded all available cases in which starchy and sugary endosperm, *Su su*, are involved together with the aleurone-factor pairs *C c*, *R r*, and *Pr pr*. The male parents of all these crosses had homozygous starchy endosperm and homozygous purple aleurone, *A C R*

TABLE 3. *Aberrant seeds maize from crosses of colorless sugary, c su and r su, by colored starchy, C R Su, and of red sugary, pr su, by purple starchy, Pr Su*

Group	Genes Concerned	Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds: All Starchy, Colored and Colorless	Number of Aberrant Seeds: All Colored, Starchy and Sugary
1........	*c su* × *C Su*...	9063– 1	260	1	
		3	240	1	
		4	320	2	
		5	179	1	1
		7	510	1	
		10178– 2	320	2	
		3	290	1	
		4	410	2	1
		5	290		1
		6	370	2	
		6	220	1	
		10179– 1	420	1	
		2	360	1	
		4	250		1
		5	380		1
		6	430	3	
		6	440	1	
		5 ears	1,750	0	0
Total....		22 ears	7,430	20	5
2........	*r su* × *R Su* ..	8572– 1	190	2	
		10185– 1	420	2	2
		2	320	1	
		4	400	2	1
		5	390		1
		6	380	1	
		7	290	2	
		7	440	1	
		10	360	2	2
		7 ears	2,720	0	0
Total....		16 ears	5,910	13	6
				All Starchy, Purple and Red	All Purple, Starchy and Sugary
3........	*pr su* × *Pr Su*	10180– 1	360		1
		5	260	1	
		7	220		1
		5 ears	710	0	0
Total....		8 ears	1,550	1	2

Pr Su, while the female parents of all had sugary endosperm, *su*. In addition, the female parents of all crosses shown in groups 1 and 2 of the table had colorless aleurone, *A c R* for group 1 and *A C r* for group 2, and those of the crosses presented in group 3 had red aleurone, *A C R pr*. In all, 47 aberrant seeds are recorded. Of these, 34 had aberrant aleurone color, 20 involving *C c Su su* (fig. 1, C), 13 *R r Su su* (fig. 1, A), and 1 *Pr pr Su su*; and 13 had aberrant endosperm texture, 5 involving *C c Su su* (fig. 1, D), 6 *R r Su su* (fig. 1, B), and 2 *Pr pr Su su*. Every one of the 34 seeds that had aberrant aleurone (colored-colorless) were starchy throughout, and all of the 13 with aberrant endosperm (starchy-sugary) had colored aleurone throughout.

In short, there have been observed (tables 2 and 3) a total of 85 aberrant seeds involving *C c* with *Su su*, *A a* with *Wx wx*, and *R r* and *Pr pr* with both *Su su* and *Wx wx*. In every case in which aleurone color was concerned, 72 in all, the aberrant spot showed the recessive color of the female parent but was invariably underlaid with the dominant corneous or starchy endosperm of the male parent; and in all of the 13 cases in which endosperm composition was concerned the aberrant spot exhibited the recessive sugary condition of the female parent but was invariably overlaid by the dominant aleurone color of the male parent. It is obvious, therefore, that for none of the 85 seeds could the aberrant spots, though they displayed in every case one or other (never both) of the two recessive maternal characters whose genes were carried in the polar nuclei, have been produced by the independent division either of one polar nucleus alone or of the two after fusion. To explain these cases on the basis of independent division of one or both polar nuclei would require the unwarranted additional assumption that in some cases—aberrant sugary spots—the polar nucleus or nuclei alone give rise to a part of the underlying endosperm but to none of the aleurone layer, while in other cases—aberrant aleurone-color spots—they give rise to a part of the aleurone but to none of the underlying endosperm. Moreover, if such behavior of independently dividing polar nuclei were so common in cases involving the endosperm factors *Wx wx* with the aleurone factors *A a*, *R r*, and *Pr pr* (table 2), and the endosperm factors *Su su* with the aleurone factors *C c*, *R r*, and *Pr pr* (table 3), why should not the same behavior of the polar nuclei be found where there is involved *Wx wx* with *C c* (table 1) or with *I i* (Collins, 1913) or *C c* with *Sh sh* (Hutchison's data)? But the facts are that in the great majority of aberrant seeds involving *C c* and *Wx wx* (55 to 3) where the aleurone layer is colorless (maternal), the underlying endosperm is waxy (also maternal), and the correspondence in outline between colorless aleurone and waxy endosperm is strikingly exact. Certainly no single hypothesis that assumes independent development of either one or both of the polar nuclei can be made to fit all the data now available.

That the somatic-mutation hypothesis suggested by the writer (1915) does not agree with the great majority of the observed facts when *C c* and

Wx wx are concerned, just as the hypotheses of independent division of polar nuclei suggested by Webber (1900) do not fit the available facts where other than these aleurone and endosperm factors are concerned, was shown earlier in this paper. The hypothesis of vegetative segregation (East and Hayes, 1911) is not sufficiently specific with respect to the mechanism of such supposed segregation to make it possible to apply crucial tests. Moreover, several cases of somatic variations often referred to as cases of vegetative segregation are quite as likely due to somatic mutation. There remains only the hypothesis of aberrant chromosome behavior (non-disjunction?) which is in accord with practically all the reported cases of aberrant endosperm development. It was shown earlier how that hypothesis fits the cases involving the linked genes *C c* (or *I i*) with *Wx wx*. That this hypothesis is not in disagreement with the cases where other endosperm and aleurone factors are concerned follows from the fact—determined by ordinary breeding tests—that these other factors are not genetically linked and that, therefore, they presumably have their loci in non-homologous chromosomes. Evidence of non-linkage for *A a* with *Wx wx* was presented by Bregger (1918), for *R r* with *Wx wx* by Kempton (1919), and for *Su su* with *C c*, *R r*, and *Pr pr* by Eyster (1921); and there is indirect evidence for *Pr pr* and *Wx wx* in Hutchison's data which show *Wx wx* to be linked with *Sh sh* and the latter to be independent of *Pr pr*. If none of these combinations of genes lies in the same chromosome, it is obvious that a non-disjunction of one chromosome could not affect more than one member of the combination, just as it is that both members of any combination lying in one chromosome must be affected by a single non-disjunction of that chromosome.

While the writer feels that the genetic evidence in favor of the hypothesis of non-disjunction, or at least of some aberrant chromosome behavior giving a similar result, as the cause of most cases of the kind of aberrant endosperm here discussed is as convincing as such evidence can well be, it is realized that direct proof must come, if at all, from cytological studies. Whether it will ever be possible to detect non-disjunction cytologically in the endosperm of maize, granting that it occurs, cannot be said. The small size of maize chromosomes and their large number, 30 in the triploid nucleus, increase the difficulty of the undertaking. Moreover, the rarity of the phenomenon lessens the chance of a successful outcome. On this latter point, however, there is this to be said: non-disjunction is doubtless a much more common occurrence than are its visible manifestations. There is no reason to suppose, for instance, that, in material of the genotype *c c C wx wx Wx*, such as that recorded in table 1, the chromosome carrying *C* and *Wx* is more often concerned in non-disjunction than are the other two homologous chromosomes each carrying *c* and *wx*. But a non-disjunction involving either of the latter could result in no visible change in either the color of the aleurone or the texture of the endosperm. It may

well be assumed, therefore, that non-disjunction within this one group of chromosomes occurs three times as frequently as it is visibly manifested in such material.

Moreover, there is no satisfactory evidence that one group of homologous chromosomes is involved more frequently than any one of the other nine groups. It may be supposed, therefore, that non-disjunction occurs on the average 30 times for every aberrant seed observed in material heterozygous for a single factor pair. Certainly non-disjunction—if such be the cause of aberrant endosperm—is not limited to the *C-I-Sh-Wx* chromosome. Aberrant endosperm has been oflserved to involve the additional aleurone and endosperm factors *A*, *R*, *Pr*, *Y*, and *Su*, all of which are inherited independently of the *C-I-Sh-Wx* group, and all of which, except possibly *R* and *Pr*, are inherited independently of one another. Since, therefore, aberrant endosperm has been observed to involve not less than five and perhaps six linkage groups, aberrant endosperm behavior is assumed to have occurred in at least the same number of groups of homologous chromosomes and there is no reason to suppose that it is not common to all ten groups.

From tables 1–3 of this paper, it is seen that 150 aberrant seeds were observed with an approximate total of 57,830 normal seeds. Of these 150 seeds, 13 involved sugary endosperm, a character that might easily be overlooked except when the aberrant spot is large. Since, moreover, the material involving sugary endosperm had to do also with an aleurone-color factor, the 13 seeds must be omitted if we are to deal with a single factor or linked-factor group, in other words, with a single chromosome, at a time. The observed ratio, when only one factor is involved, is 57830 : 137, or approximately one aberrant case in every 423 seeds. If now it be assumed that non-disjunction occurs thirty times as frequently as aberrant seeds in such material, non-disjunction should occur on the average once in about 14 seeds. In more than half of the aberrant seeds reported in this paper (77 out of 150) the aberrant part included approximately one sixth or more of the surface area of the seed and in about one twelfth of them it included more than one half of the seed. Consequently, non-disjunction must occur, if at all, fairly early in endosperm development in a considerable percentage of cases. It would seem worth while, therefore, for cytologists to search for it at least in the early divisions of the endosperm nucleus.

An observation noted earlier in this paper suggests that irregularities besides non-disjunction may occur in endosperm development. It was noted that 8 out of 65 aberrant seeds involving *C c* and *Wx wx* were mottled, exhibiting numerous small spots of colorless aleurone instead of a single spot. One of these mottled seeds was so immature that the endosperm texture could not be determined, but in the other seven the colorless spots were underlaid by waxy endosperm (fig. 1, L), this association indicating definitely aberrant chromosome behavior. It does not seem likely that non-disjunction would occur repeatedly in the development of a single

seed, but if it does not there must have been very irregular migration of endosperm nuclei after non-disjunction occurred. In material involving *C c Su su* and *A a Wx wx*, 6 of the 22 seeds showing aberrant aleurone color were mottled. Practically all of the normal seeds in material where *R r* is involved were mottled, and the aberrant seeds showed mottling in the colored part (fig. 1, A and E), but mottling is known to occur commonly in aleurone heterozygous for *R* when *R* enters with the sperm and *r r* with the polar nuclei and, whatever its ultimate cause, it is not to be confused with what is here termed aberrant endosperm.

Summary

It has been shown that, when aberrant seeds occur in crosses in which recessive aleurone and endosperm characters are contributed by the female parent and the corresponding dominant characters by the male parent, spots of the recessive (maternal) aleurone color are in the great majority of cases underlaid by the recessive (maternal) type of endosperm if the genes for these aleurone and endosperm characters are genetically linked, as shown by breeding tests, while similar recessive aleurone-color spots are always, so far as observed, underlaid by the dominant (paternal) type of endosperm and recessive endosperm parts are overlaid by the dominant aleurone color if the aleurone-color and endosperm-composition genes are not linked.

These facts are held to support the hypothesis of occasional aberrant chromosome behavior—possibly non-disjunction—and are incompatible with the earlier hypotheses involving failure of normal fusion of the second sperm nucleus with the polar nuclei, and also make untenable, except in rare cases, the hypothesis of somatic mutation.

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